Erythropoietin is equally effective as fresh-blood transfusion at reducing infarct size in anemic rats

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Objective: **We recently demonstrated that transfusion of anemic animals up to 100 g/L hemoglobin with fresh blood protects the heart from ischemic injuries following myocardial infarction. Erythropoietin has cardioprotective effects independent of its erythropoietic activity. The objective of this study was to compare the cardioprotective effects of erythropoietin treatment to fresh-blood transfusion in anemic rats after acute myocardial infarction.**

Design: **Randomized animal study.**

Setting: **University laboratory.**

Subjects: **Male Sprague-Dawley rats weighing 200–300 g.**

Intervention: **Myocardial infarction was induced by coronary artery ligation in 76 rats, 55 of which were anemic (80–90 g/L) and 21 of which had normal hemoglobin levels. Animals were randomized to erythropoietin (2000 units/kg), fresh-blood transfusion to 100 g/L hemoglobin, or saline-treatment groups immediately following myocardial infarction.**

Measurements and Main Results: **At 24 hrs after myocardial infarction, cardiac function and infarct size were determined. Myocardial apoptosis was determined by caspase-3 activity and terminal deoxynucleotidyl transferase d-UTP nick end labeling (TUNEL) assay. Infarct size was significantly decreased in anemic rats treated with erythropoietin or blood transfusion compared to those in the saline-treatment group. Cardiac function, as measured by maximal positive and minimal negative first derivatives of left ventricular pressure, was better preserved in the normal hemoglobin groups and the erythropoietin- or transfusion-treated anemic animals compared to saline-treated anemic animals. Myocardial caspase-3 activity and TUNEL-positive nuclei were significantly increased in anemic rats but were decreased by erythropoietin treatment or red blood cell transfusion.**

Conclusions: **Erythropoietin treatment is equally effective as fresh-blood transfusion in anemic rats after acute myocardial infarction at reducing infarct size, myocardial apoptosis, and improving cardiac function. (Crit Care Med 2010; 38:2215–2221)**

KEY WORDS: anemia; blood transfusion; myocardial infarction; erythropoietin

Anemia is a common condition in patients suffering from acute myocardial infarction (MI). The estimated prevalence of anemia, defined as a hemoglobin (Hb) level of <120 g/L, is approximately 15–40% in patients with acute MI. Elderly populations show increased rates with more severe anemia (Hb <100 g/L) presenting in approximately 5% of individuals (1, 2). Anemia and the resulting decrease in the oxygen-carrying capacity of the blood could increase the potential for tissue damage during acute MI, and a number of clinical studies suggest that anemia adversely affects clinical outcomes (2–5). In the largest of these studies, Sabatine et al (2) found that the mortality rate increased in acute coronary syndromes as Hb levels decreased below 14 g/dL, and there was an adjusted odds ratio of 1.21 (95% confidence interval, 1.12–1.30) for each 10 g/L decrement in Hb. Given the prevalence of anemia and its direct adverse effects on cardiovascular function, it is important to seek effective therapeutic interventions to reduce myocardial damage after acute MI (6, 7).

Presently, the most common method of treating anemic patients is by red blood cell (RBC) transfusion. The effects of transfusion on acute coronary syndromes have also been studied, and there have been conflicting results. In a study conducted by Wu et al (1), transfusion was shown to reduce short-term mortality in elderly patients after MI; however, contradictory results associating transfusion with higher mortality rates were observed in the setting of acute coronary syndromes (8). A subsequent clinical study found that although transfusion with a nadir Hb level of >80 g/L was associated with an increased mortality rate in patients experiencing acute MI, transfusion in patients with a nadir Hb level of <80 g/L showed a protective effect following MI (9). Our group recently found similar results in a rat model showing transfusion to a Hb level of 100 g/L to be cardioprotective, but transfusion to higher Hb levels did not seem to be beneficial (10). If blood transfusion is to become an accepted treatment for myocardial ischemia, then the optimal Hb level for transfusion must be determined and balanced against the risks such as volume overload, transfusion-related acute lung injury, infection, and immunosuppressive effects associated with this intervention.

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This study was supported by the CIHR, Canadian Blood Services, Hema Quebec, Bayer Partnership Fund, and the Anemia Institute for Research and Education. Dr. Feng is a Heart and Stroke Foundation of Ontario Career Investigator.

Dr. Xenocostas has been a consultant for and has received educational grants and honoraria from Ortho Biotech, Canada. Dr. I Chin-Yee is the recipient of a grant from Canadian Blood Services. The remaining authors have not disclosed any potential conflicts of interest.

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DOI: 10.1097/CCM.0b013e3181f17d6e

Potential alternatives that avoid the risks of transfusion can include cytoprotective agents administered to blunt postischemic injury. Erythropoietin (EPO) is a 34-kDa glycoprotein that promotes survival of erythroid progenitors and thereby maintains the circulating RBC mass. In addition, pharmacologic doses of EPO have been shown to produce cytoprotective effects through interaction with the erythropoietin receptor, which is located in a variety of tissues in the cardiovascular, renal, gastrointestinal, and central nervous systems (11, 12). In the ischemic myocardium, EPO has both immediate and delayed protective effects including the inhibition of cardiomyocyte apoptosis (13–16), reduction of inflammatory response to reperfusion (17, 18), improvement of tissue microcirculation (19), antiarrhythmic effects (20, 21), and stimulation of angiogenesis (22–25).

Currently, it is unknown whether EPO can produce similar cardioprotective effects during MI in an anemic host or how these effects compare to improved outcomes observed with fresh-blood transfusion. To more clearly define the effects of anemia and blood transfusion during acute MI we developed an animal model in which anemia both increased infarct size and decreased cardiac function and animal survival, while blood transfusion reduced infarct size and improved cardiac function (10). In the present study, we investigated the cardioprotective effects of EPO in anemic animals compared to animals that received transfusion to explore potential alternative cardioprotective treatment modalities.

MATERIALS AND METHODS

Experimental Animals and Induction of Anemia. The experiments were conducted by using male Sprague-Dawley rats (200–300 g). All animals were provided water and food *ad libitum* and housed in a temperature- and humidity-controlled facility with 12-hr light and dark cycles. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). Experimental protocols were approved by the University of Western Ontario Animal Use Subcommittee.

To induce anemia, rats were fed an iron-deficient diet (10–20 ppm; TestDiet 5859, Richmond, IN) previously described by Strube et al (26) in combination with phlebotomy (2–3 mL) from the jugular vein twice weekly to reach target Hb levels of 80–90 g/L. Immediately following phlebotomy, rats were infused with an equal volume of 10% pentastarch (Bristol-Myers Squibb, Montreal, Quebec, Canada).

Experimental Design and Transfusion. Rats were randomly assigned to five experimental groups:

Group 1. Normal Hb level at 140–150 g/L, MI with saline (vehicle) treatment (n = 11)
Group 2. Normal Hb level at 140–150 g/L, MI with EPO treatment (n = 10)
Group 3. Hb level at 80–90 g/L, MI with saline (vehicle) treatment (n = 20)
Group 4. Hb level at 80–90 g/L, MI with EPO treatment (n = 18)
Group 5. Hb level at 80–90 g/L, MI with blood transfusion to Hb level of 100 g/L (n = 17)

To achieve cardioprotective effects, high doses of EPO (2000–5000 units/kg IV) are commonly used and have been shown to be effective in rodents following MI (11, 21). Therefore, in the present study, rats in groups 2 and 4 were treated with IV EPO (2000 units/kg) via jugular vein immediately after coronary artery ligation surgery, and 0.1 mL of saline (vehicle) was given to control animals in groups 1 and 3. Transfusion was performed in group 5 immediately after coronary artery ligation surgery to raise the Hb level to 100 g/L by using fresh blood (stored <4 hrs). Donor blood was collected from rats and stored as previously described (10). Briefly, rats were anesthetized with pentobarbital (65 mg/kg IP), and the carotid artery was cannulated to retrieve the maximum amount of blood. Blood was collected into a 20-mL syringe with 3 mL of citrate-phosphate-dextrose-adenine-1 (Baxter, Toronto, Ontario, Canada) and injected into a sterile blood-collection bag (Fenwal, Baxter). The whole blood was kept at 4°C for less than 4 hrs. Right before transfusion, the stored nonleukoreduced blood was drawn from the collection bag and centrifuged at 500*g* for 5 mins at 4°C. The supernatant was removed to achieve a hematocrit level of 75%. Total volume of packed RBCs infused to achieve 100 g/L Hb was approximately 3 mL within 30 mins. Thirty minutes after transfusion, the Hb levels in the recipient rats were measured from a 10-μL blood sample obtained from the saphenous vein by spectrophotometry using a Hb assay kit (Pointe Scientific, Canton, MI).

Induction of MI. MI was induced by ligation of the left descending coronary artery as described in our previous reports (10, 27). Briefly, rats were anesthetized with an intramuscular injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). Animals were then intubated and artificially ventilated. A left thoracotomy was performed to expose the heart, and the left anterior descending coronary artery was ligated by positioning a 6–0 silk suture between the pulmonary artery out-flow tract and the left atrium. The lungs were thereafter hyperinflated by using positive end-expiratory pressure, and the thorax immediately closed. Animals were caged individually after each surgical operation.

Hemodynamic and Infarct Size Measurements. Hemodynamic measurements were made to assess *in vivo* cardiac function and were performed as previously described 24 hrs after coronary artery ligation (10). Rats were reanesthetized with ketamine and xylazine, and a polyethylene catheter (PE-50) was inserted into the right carotid artery to record the arterial pressure. The catheter was then advanced retrograde into the left ventricle (LV) for recording of LV pressures. Pressure signals were fed to an analog-digital converter and collected by a computer. Heart rate, arterial pressure, LV systolic pressure, LV end-diastolic pressure, and maximal positive and minimal negative first derivatives of LV pressure ($+dP/dt_{max}$ and $-dP/dt_{min}$; parameters that represent contractility and diastolic relaxation, respectively) were analyzed by PowerLab Chart software (ADInstruments, Colorado Springs, CO).

Infarct size was determined according to our previous reports (10, 21). Briefly, after hemodynamic measurements, 3 mL of Evans blue dye (2% in saline) was injected into the ascending part of the aorta to stain the area of the myocardium perfused by the patent coronary arteries, thereby delineating the area at risk by negative staining. The heart was excised, and the LV was carefully isolated and sliced transversely into sections 3–4 mm thick. Sections were weighed and incubated in 5% triphenyltetrazolium chloride (TTC) in phosphate-buffered saline at 37°C for 15 mins to stain the viable myocardium. Photographs of heart slices were taken with a digital camera. The nonrisk area (stained by Evans blue), risk area (unstained by Evans blue), and infarct area (unstained by TTC) were quantitated by using an image-analysis system (Sigma ScanPro, Ashburn, VA). By using volumetric analyses, the proportions of the LV that were normally perfused, at risk, or infarcted were calculated and expressed as a percentage of total LV weight. Infarct size (%) was expressed as the weight of infarcted LV divided by the weight of LV at risk.

Caspase-3 Activity Measurement. Caspase-3 activity was measured by using a caspase-3 assay kit AK-700 (BIOMOL, Plymouth Meeting, PA) as we previously described (27–29). Briefly, heart tissues from the peri-infarct area were homogenized, and protein concentration was determined by Lowry assay. Samples (200 μg of protein) were incubated at 37°C for 4 hrs in the presence of the caspase-3 substrate. N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin with or without inhibitor N-acetyl-Asp-Glu-Val-Asp-CHO. Fluorescent intensity (excitation at 360 nM, emission at 460 nM) was measured by using a fluorescent microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA). Data

Table 1. Body weight and hemoglobin at baseline and following 2 wks of treatment with iron-deficient diet in combination with bleeding before coronary artery ligation surgery in 5 experimental groups

Group	Body Weight, g		Hemoglobin, g/L	
	Baseline	Before Surgery	Baseline	Before Surgery
1. Hb 140–150g/L, saline (n = 11)	182.1 ± 2.3	267.8 ± 2.8	142.6 ± 1.2	143.3 ± 1.3
2. Hb 140–150g/L, erythropoietin (n = 10)	178.4 ± 1.8	268.9 ± 3.3	146.0 ± 1.1	146.6 ± 0.9
3. Hb 80–90g/L, saline (n = 20)	181.8 ± 4.8	269.1 ± 6.7	149.9 ± 1.8	84.4 ± 0.8
4. Hb 80–90g/L, erythropoietin (n = 18)	182.1 ± 1.4	271.8 ± 2.8	147.1 ± 1.0	83.1 ± 1.2
5. Hb 80–90g/L, red blood cells (n = 17)	181.3 ± 2.4	274.4 ± 2.8	145.4 ± 1.3	82.5 ± 0.9

Hb, hemoglobin.

Data are mean ± standard error. Red blood cells indicate packed red blood cell transfusion to reach Hb of 100 g/L. Body weight was not statistically different between groups. Hemoglobin levels were not significantly different within the normal or anemic groups.

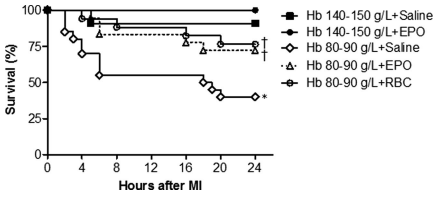

Figure 1. Effects of erythropoietin (*EPO*) treatment and transfusion on survival in anemic rats after myocardial infarction (*MI*). Recombinant human erythropoietin (2000 units/kg) was injected intravenously into the tail vein immediately after MI. Transfusion was performed in rats with hemoglobin (*Hb*) levels of 80–90 g/L immediately after MI to raise Hb levels to 100 g/L using fresh-packed red blood cells (*RBCs*). Survival was monitored for 24 hrs following surgery. n = 10–20 rats per group, * $p < .05$ vs. Hb 140–150 g/L + saline or EPO; † $p < .05$ vs. Hb 80–90 g/L + saline.

were expressed as the amount of 7-amino-4-methylcoumarin substrate cleaved per μg protein per hour.

TUNEL Staining. Terminal deoxynucleotidyl transferase d-UTP nick end labeling (TUNEL) staining was performed by using an In Situ Cell Death Detection kit (Roche, Indianapolis, IN) on paraffin heart sections (5 μm) as previously described (29–31). Hoechst 33,342 was used as a counterstain. Fifteen separate fields of peri-infarct area were examined by using a fluorescent microscope (Observer D1, Zeiss, Gottingen, Germany) at ×630 magnification to quantify the number of TUNEL-positive nuclei. Images were obtained by using a laser confocal microscope (LSM 510 Meta, Zeiss). Data were expressed as the average percentage of TUNEL-positive nuclei by two independent observers.

Statistics. Data are expressed as the means ± SEM. One-way analysis of variance was performed followed by Bonferroni test. p values of <.05 were considered statistically significant.

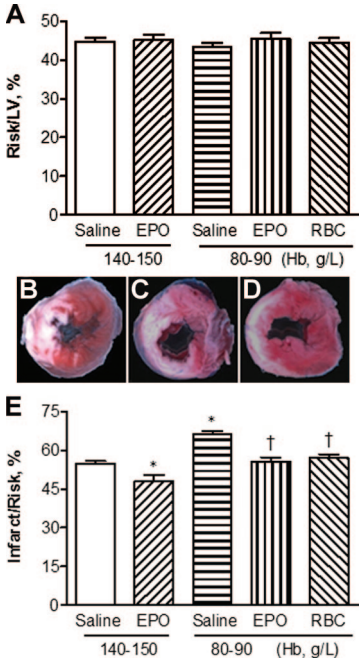

Figure 2. Effects of erythropoietin (*EPO*) treatment and packed red blood cell (*RBC*) transfusion on infarct size after myocardial infarction in anemic rats. *A*, Percent weight of risk area to total left ventricular (*LV*) weight shows similar ischemic areas between all groups; *B–D*, Representative heart sections with triphenyltetrazolium chloride staining after myocardial infarction from normal hemoglobin (*Hb*) + saline (*B*), Hb 80–90 g/L + saline (*C*), and Hb 80–90 g/L + EPO (*D*) treatment groups; *E*, Infarct size expressed as a percentage of weight of infarct to weight of LV at risk. Data are mean ± SEM from 7–10 rats per group. * $p < .01$ vs. Hb 140–150 g/L saline; † $p < .01$ vs. Hb 80–90 g/L saline.

RESULTS

Body Weight and Hb. All animals had a similar body weight and Hb level at baseline (Table 1). Animals fed an iron-deficient diet in combination with phlebotomy had significantly decreased Hb levels compared to those in the standard-diet group by the end of a 2-wk period before surgery; mean Hb levels reached the target level of 80–90 g/L. Following transfusion, the Hb level increased to 101.0 ± 0.7 g/L in anemic animals. EPO treatment for 24 hrs did not change Hb levels in either normal (139.0 ± 3.0 vs. 139.5 ± 4.0 g/L, p = not significant [NS]) or anemic (85.7 ± 2.0 vs. 86.3 ± 1.5 g/L, p = NS) groups.

Effects of EPO and Transfusion on Survival After MI. Following MI, the 24-hr survival rate was monitored. In the normal Hb group (140–150 g/L) survival after MI in rats treated with or without EPO was 100% (10/10) and 91% (10/11), respectively (Fig. 1). In the anemic groups (Hb 80–90 g/L), animals treated with saline (vehicle without EPO) demonstrated a significantly decreased survival rate after MI 40% (8/20) compared to the normal Hb group ($p < .05$). The survival rate improved significantly after EPO treatment in the Hb 80–90 g/L groups (72% [13/18]) 24 hrs after MI compared to saline-treated anemic rats (40% [8/20], $p < .05$; Fig. 1).

To study whether blood transfusion had any effect on survival, packed RBCs were immediately transfused following MI. The survival rate was significantly increased after transfusion to raise the Hb level from 80–90 g/L to 100 g/L (77% [13/17]) compared to anemic rats receiving saline (40% [8/20], $p < .05$; Fig. 1). Furthermore, survival rates were similar in the blood-transfused (77%) and EPO-treated groups (72%, p = NS; Fig. 1).

Effects of EPO and Transfusion on Infarct Size After MI in Anemic Rats. Twenty-four hours after MI, the area at risk to LV weight ratios were not significantly different between any groups, indicating that a similar degree of myocardial ischemia was induced in all groups (p = NS; Fig. 2*A*). However, the infarct to risk weight ratios were significantly increased in the Hb 80–90 g/L saline-treated group compared to the normal Hb groups ($p < .01$; Fig. 2*E*). Infarct size was significantly decreased in the EPO-treated compared to saline-treated rats in both the Hb 140–150 g/L and Hb 80–90 g/L groups. Furthermore, RBC transfusion significantly decreased infarct size compared to the Hb 80–90 g/L saline-treated group ($p < .01$; Fig. 2*E*). However, no statistical difference was observed between the EPO-

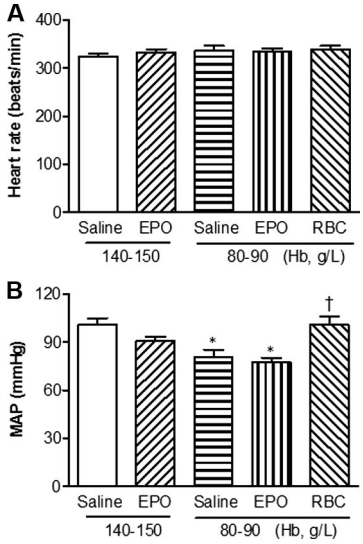

Figure 3. Effects of erythropoietin (*EPO*) treatment and transfusion on heart rate (*A*) and mean arterial pressure (*MAP*) (*B*) 24 hrs after myocardial infarction in anemic rats. Data are mean ± SEM from 7–10 rats per group. *RBC*, red blood cell. * $p < .05$ vs. hemoglobin (*Hb*) 140–150 g/L + saline; † $p < .01$ vs. Hb 80–90 g/L + EPO.

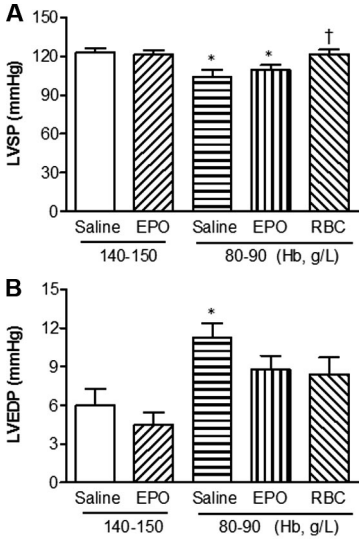

Figure 4. Effects of erythropoietin (*EPO*) treatment and transfusion on left ventricular (*LV*) pressures 24 hrs after myocardial infarction in anemic rats. *A*, Left ventricular systolic pressure (*LVSP*); *B*, Left ventricular end diastolic pressure (*LVEDP*). Data are mean ± SEM from 7–10 rats per group. *RBC*, red blood cell. * $p < .05$ vs. hemoglobin (*Hb*) 140–150 g/L + saline; † $p < .05$ vs. Hb 80–90 g/L + saline.

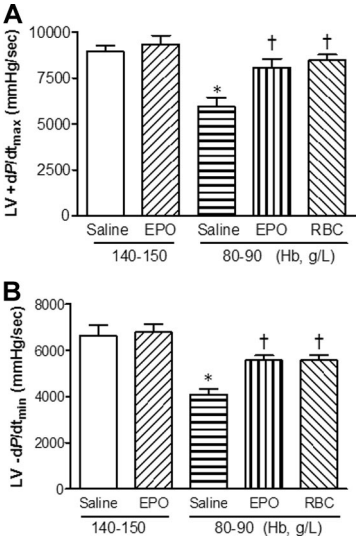

Figure 5. Effects of erythropoietin (*EPO*) treatment and transfusion on left ventricular (*LV*) contractility (+d*P*/dt) and relaxation (−d*P*/dt) 24 hrs after myocardial infarction in anemic rats. *A*, LV + dP/dt_{max}; *B*, LV + dP/dt_{min}. Data are mean ± sem from 7–10 rats per group. *RBC*, red blood cell. * $p < .01$ vs. hemoglobin (*Hb*) 140–150 g/L + saline or EPO; † $p < .01$ vs. Hb 80–90 g/L + saline.

treated and transfused groups of anemic rats (p = NS; Fig. 2*E*).

Effects of EPO and Transfusion on Cardiac Function After MI in Anemic Rats. Cardiac function was determined 24 hrs after MI. Heart rate was not significantly different between all groups (p = NS; Fig. 3*A*). The mean arterial pressure was significantly decreased in the anemic groups without blood transfusion compared to the normal Hb groups ($p < .01$; Fig. 3*B*). However, the mean arterial pressure was significantly increased in the RBC-transfused group compared to the saline-treated group ($p < .01$; Fig. 3*B*). LV systolic pressure was decreased in the Hb 80–90 g/L saline-treated group compared to the normal Hb saline-treated group ($p < .01$). RBC transfusion significantly increased LV systolic pressure compared to the Hb 80–90 g/L saline-treated group ($p < .01$; Fig. 4*A*). LV end-diastolic pressure was increased only in the Hb 80–90 g/L saline-treated group compared to the normal Hb saline- or EPO-treated group ($p < .01$). However, the LV end-diastolic pressure in the EPO-treated and RBC-transfused groups was not significantly different when compared to their respective controls (p = NS; Fig. 4*B*).

Reduction in Hb levels from 140–150 to 80–90 g/L after MI correlated with a significant decrease in LV $+dP/dt_{max}$ (contractility) and $-dP/dt_{min}$ (diastolic function) in saline-treated rats ($p < .01$; Fig. 5). EPO treatment in anemic rats significantly increased LV $+dP/dt_{max}$ ($p < .01$) and $-dP/dt_{min}$ ($p < .05$) compared to anemic rats treated with saline. Fresh RBC transfusion to raise Hb levels to 100 g/L significantly increased LV $+dP/dt_{max}$ and $-dP/dt_{min}$ compared to the saline-treated group ($p < .01$; Fig. 5). There was no significant difference in LV $+dP/dt_{max}$ or $-dP/dt_{min}$ between the EPO-treated and RBC-transfused groups (p = NS). Central venous pressure was monitored by placing a PE-50 into the superior vena cava via the right jugular vein before and 1 hr after transfusion. Central venous pressure was not significantly altered by RBC transfusion (4.8 ± 1.0 vs. 5.6 ± 0.6 mm Hg, n = 5, p = NS).

Effects of EPO and Transfusion on Myocardial Apoptosis After MI in Anemic Rats. To assess possible mechanisms by which EPO and RBC transfusion improve the outcome of these animals, myocardial apoptosis was determined. Caspase-3 activity in the peri-infarct area was significantly increased in anemic rats compared to that in normal Hb rats ($p < .05$; Fig. 6*A*). EPO treatment and fresh RBC transfusion significantly decreased the myocardial caspase-3 activity compared to their respective controls ($p < .05$; Fig. 6*A*). However, there was no significant difference between EPO treatment and fresh RBC transfusion in anemic rats (p = NS; Fig. 6*A*).

Myocardial apoptosis was further analyzed by using TUNEL staining. Representative images of TUNEL staining for normal Hb and saline, Hb 80–90 g/L and saline, and Hb 80–90 g/L and EPO treatment groups are shown in Figure 6*B*, 6*C*, and 6*D*, respectively. Our data showed that the number of TUNEL-positive nuclei was significantly increased in the peri-infarct area of the anemic rats compared to the Hb 140–150 g/L group ($p < .05$; Fig. 6*E*). EPO treatment in both normal and anemic rats, and fresh RBC transfusion to raise Hb levels to 100 g/L, significantly decreased TUNEL-positive nuclei compared to their respective saline controls ($p < .05$; Fig. 6*E*). Consistent with the caspase-3 data, there was no significant difference between EPO treatment and fresh RBC transfusion in anemic rats (p = NS; Fig. 6*E*).

DISCUSSION

The present study demonstrated for the first time in an anemic rat model that EPO treatment independent of erythropoiesis is cardioprotective following acute MI, and that the beneficial effects are similar in magnitude compared to those

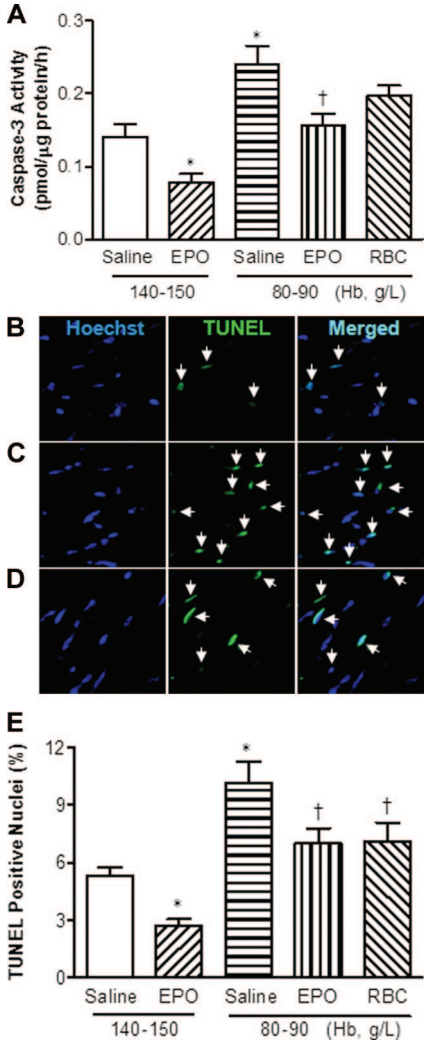

Figure 6. Effects of erythropoietin (*EPO*) treatment and transfusion on myocardial apoptosis in peri-infarct area 24 hrs after myocardial infarction in anemic rats. *A*, Myocardial caspase-3 activity; *B–D*, Representative confocal images of terminal deoxynucleotidyl transferase d-UTP nick end labeling (*TUNEL*) staining from normal hemoglobin (*Hb*) + saline (*B*), Hb 80–90 g/L + saline (*C*), and Hb 80–90 g/L + EPO-treated (*D*) groups. TUNEL-positive nuclei stained in green with Hoechst (blue) as a nuclear stain. White arrows indicate positive signal. *E*, Quantitative analysis of TUNEL-positive nuclei. Data are mean ± SEM, n = 5 rats per group. *RBC*, red blood cell. * $p < .05$ vs. Hb 140–150 g/L + saline; † $p < .01$ vs. Hb 80–90 g/L + saline.

observed after RBC transfusion in which the Hb level was increased by 20 g/L to a target Hb level of 100 g/L. Both EPO treatment and RBC transfusion increased animal survival and decreased myocardial apoptosis and infarct size after MI. Cardiac function as measured by rate of LV contraction and relaxation was also significantly improved in both the EPO and transfusion groups.

EPO induces cardioprotective effects during ischemia in animals with normal Hb levels via a number of mechanisms independent of erythropoeisis (11, 21, 31). EPO binds to the EPO receptor and subsequently activates the PI3-kinase/Akt pathway, leading to reduction of cardiomyocyte apoptosis (11, 13, 30, 31). EPO also improves skeletal muscle microcirculation in sepsis (19) and reduces ventricular fibrillation during myocardial ischemia and reperfusion (20). We further demonstrated that the antiarrhythmic effects are mediated by increased expression of neuronal nitric oxide synthase following EPO treatment (21). Finally, the beneficial effects of EPO are also mediated by its anti-inflammatory properties to reduce cytokines and oxidative stress (17, 18, 32). In the present study, we demonstrated that EPO treatment decreased myocardial apoptosis, therefore increasing the number of surviving cardiomyocytes and preserving myocardial function. In anemic animals without EPO treatment this effect was absent, which resulted in reduced myocardial function.

The magnitude of the protective effects of EPO in the absence of any changes in Hb is striking in our study, because it was similar to the effects seen when Hb was increased by 20 g/L. Although we saw beneficial effects from both EPO treatment and transfusion, we did not test both treatments together to determine if there could be additive or synergistic effects for further improvement in outcomes. However, the differences in outcomes between the EPO-treated group, the transfused rats, and the control nonanemic animals were small. It could be argued that both treatments used in isolation produced near-maximal salvaging of myocardium at risk in this model of irreversible ischemia. There are limits to the amount of myocardium that can be salvaged after irreversible ischemia, and it is unlikely that combining both treatments would be able to improve outcomes further in this model of MI.

Although transfusion remains the most common therapeutic intervention for the immediate correction of anemia, its effectiveness is controversial (1, 8, 9), and it is associated with a number of risks including volume overload, transfusion-related acute lung injury, infection, and immunosuppressive effects (33). EPO treatment avoids these risks because the volume administered is negligible and there is no exposure to alloantigens or cytokines. However, potential drawbacks of EPO might include the activation of platelets with an increased incidence of thrombosis (34)—an adverse effect that has to be considered even in the context of antiplatelet therapy used as part of the standard treatment for acute MI.

STUDY LIMITATIONS

1. The model used was one of irreversible ischemia, and we did not examine the protective effects of EPO following reperfusion injury. In the clinical setting, patients with acute coronary syndromes are commonly treated by thrombolysis or angioplasty. However, the protective effects of EPO seem to extend to reperfusion models as well (14, 23, 31). Furthermore, because our experiment only measured outcomes 24 hrs following MI, long-term effects were not examined. In other studies EPO treatment was shown to reduce infarct size, promote angiogenesis, and improve cardiac function 4 to 9 wks after MI (23, 24).
2. The anemic group was transfused to only one Hb level of 100 g/L, because our previous study found that a target Hb level of 100 g/L was optimal for increased animal survival, decreased infarct size, and improved cardiac function (10). Clinically, transfusion to a Hb level of >80 g/L failed to show any benefits and actually seemed to be deleterious (9, 35, 36). Therefore, extrapolation from the current animal study to the human population in terms of transfusion threshold should be cautioned. In addition, to minimize RBC-storage lesions, blood was stored less than 4 hrs before transfusion in the present study. However, the use of 4-hr fresh blood may not be possible in clinical practice.
3. Central venous pressure is commonly used to assess cardiac preload and volume status (37). In the present study, volume overload was not observed based on central venous pressure measurements during blood transfusion over a period of 30 mins. It is important to note that changes in central venous pressure are affected by the speed of fluid infusion and compliance of the cardiovascular system (37). Thus, central venous pressure measurements alone may not accurately reflect blood volume changes (38).
4. β-Adrenergic blockers have been shown to improve coronary perfusion

reserve, promote the growth of coronary arterioles, and facilitate regional myocardial perfusion in rat MI models (39). Because β-blockade is a standard treatment strategy in patients with MI, further studies using β-blockers in combination with EPO or transfusion are warranted.

5. Induction of an iron-deficient state in the anemic animals itself may have influenced cardiac function (40). However, our iron-deficient rats undergoing treatment demonstrated similar growth and hemodynamics compared to control rats. It is also clinically relevant to note that a significant proportion of patients with acute coronary syndromes are anemic, and although the anemia may not be due to iron deficiency, ongoing inflammatory stress can contribute to anemia of chronic disease resulting in a functional iron-deficiency state (41).

CONCLUSIONS

A novel finding from our study is that the cardioprotective effects of EPO can be extended to the anemic host—an important consideration given the significant number of patients who have acute coronary syndrome and are anemic. Furthermore, EPO was equally effective as fresh-blood transfusion at reducing infarct size and improving cardiac function, and these effects occurred in the absence of any changes in Hb. Our findings suggest that use of EPO may offer a new treatment strategy for anemic patients experiencing acute MI. The demonstration of clinical benefits of EPO requires further clinical study and awaits the results of ongoing trials.

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